

Production of Glutathionyl and Protein-derived Radicals in Macrophages and Erythrocytes Treated with Peroxynitrite

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Peroxynitrite (PN) which is formed by the fast reaction between nitric oxide and superoxide anion, has been receiving increasing attention as a mediator of human diseases and as a toxin against microorganisms. An initial controversy about the possibility of radical production from PN in test tubes has been resolved and presently, it is important to establish whether PN produces free radicals in biological environments. Relevantly, EPR studies have demonstrated the production of biomolecule-derived radicals upon PN addition to human plasma [1,2], human erythrocytes [3] and a macrophage cell line (J774) [4]. Low PN concentrations added to plasma produced the ascorbyl, albumin-thiyl and an urate-derived radical [1]. In erythrocytes, production of a hemoglobin-tyrosyl radical has been demonstrated by spin trapping experiments with a nitroso spin trap [3]. In macrophages, a nitroso trap was also used to detect protein-tyrosyl radicals [4]. The use of a nitron spin trap (DMPO), however, led to the detection of the glutathionyl radical [4]. These results led us to re-examine the interaction of PN with erythrocytes in the presence of DMPO.

It was indeed possible to detect the DMPO-glutathionyl radical adduct in incubations of PN (0.2-1.0 mM) with erythrocytes (10-50%). With higher PN concentrations (= 2 mM), the dominant EPR signal was due to a DMPO-hemoglobin radical adduct. Similar results were obtained with hemolysates but signal intensities were significantly higher for the same PN concentration. Both, the DMPO-glutathionyl and the DMPO-hemoglobin radical adduct were detectable when DMPO was added from 2 to 5 min after PN, indicating that the radicals are produced from secondary reactions. Indeed, kinetic measurements of GSH depletion showed that its level keep decreasing up to 10 min after PN addition and then, starts to recover. Taken together with published studies [3,5 & others], our results indicate that oxyhemoglobin is oxidized by PN to produce ferryl-hemoglobin. This species decays by several routes, including the oxidation of intracellular glutathione to the glutathionyl radical, and oxidation of its own aminoacid residues to produce hemoglobin-tyrosyl and hemoglobin-thiyl radical. The results will be discussed in the context of the bioregulatory and biodamaging effects of peroxynitrite.

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